



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(21) International Application Number: PCT/EP97/00211</p> <p>(22) International Filing Date: 17 January 1997 (17.01.97)</p> <p>(30) Priority Data:  96870005.4 26 January 1996 (26.01.96) EP  (34) Countries for which the regional or international application was filed: AT et al.  96870081.5 25 June 1996 (25.06.96) EP  (34) Countries for which the regional or international application was filed: AT et al.</p> <p>(71) Applicant (for all designated States except US): INNOGENET-ICS N.V. (BE/BE); Industriepark Zwijnaarde 7, P.O. Box 4, B-9052 Ghent (BE).</p> <p>(72) Inventors; and  (75) Inventors/Applicants (for US only): STUYVER, Lieven (BE/BE); Holestraat 8, B-9552 Herzele (BE). LOUWAGIE, Joost (BE/BE); Melselestraat 45, B-2070 Zwijndrecht (BE). ROSSAU, Rudi (BE/BE); Wilgheovestraat 45, B-2180 Ekeren (BE).</p> <p>(74) Agent: DE CLERCQ, Ann; Innogenetics N.V., Industriepark Zwijnaarde 7, P.O. Box 4, B-9052 Ghent (BE).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b>  <i>With international search report.  Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
(54) Title: METHOD FOR DETECTION OF DRUG-INDUCED MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE		
<p>(57) Abstract</p> <p>The present invention relates to a method for the rapid and reliable detection of drug-induced mutations in the reverse transcriptase gene allowing the simultaneous characterization of a range of codons involved in drug resistance using specific sets of probes optimized to function together in a reverse-hybridization assay. More particularly, the present invention relates to a method for determining the susceptibility to antiviral drugs of HIV strains present in a biological sample, comprising: (i) if need be releasing, isolating or concentrating the polynucleic acids present in the sample; (ii) if need be amplifying the relevant part of the reverse transcriptase genes present in said sample with at least one suitable primer pair; (iii) hybridizing the polynucleic acids of step (i) or (ii) with at least two RT gene probes hybridizing specifically to one or more target sequences with said probes being applied to known locations on a solid support and with said probes being capable of simultaneously hybridizing to their respective target regions under appropriate hybridization and wash conditions allowing the detection of homologous targets, or said probes hybridizing specifically with a sequence complementary to any of said target sequences, or a sequence wherein T is replaced by U; (iv) detecting the hybrids formed in step (iii); (v) inferring the nucleotide sequence at the codons of interest and/or the amino acids of the codons of interest and/or antiviral drug resistance spectrum, and possibly the type of HIV isolates involved from the differential hybridization signal(s) obtained in step (iv).</p>		

**Figure 1:** Natural and drug-induced variability in the vicinity of codons 41, 50, 67-70, 70-75, 151, 181-184, 215 and 219 of the HIV RT gene.

Region I

38	39	40	41	42	43	44
TGT	ACA	GAA	ATG	GAA	AAG	GAA
		G	T	G	A	
			C		G	

Region II

47	48	49	50	51	52	53
ATT	TCA	AAA	ATT	GGG	CCT	GAA
		G	G			
			C			
			C			

Region III

65	66	67	68	69	70	71	72
AAA	AAA	GAC	AGT	ACT	AAA	TGG	AGA
G	G	A		A	G		
				A	G		
				GA			
				G			

Region IV

73	74	75	76	77
AAA	TTA	GTA	GAT	TTC
G	G	G	C	
		AC		

Region V

148	149	150	151	152	153	154
GTG	CTT	CCA	CAG	GGA	TGG	AAA
	C		AT			
	G	G	A			
			T			

Region VI

180	181	182	183	184	185	186	187
ATC	TAT	CAA	TAC	ATG	GAT	GAT	TTA
		G	T	A	C	G	GG
				G			G
	G			G			



Region VII

212	213	214	215	216
TGG	GGA	TTT	ACC	ACA
	G	C	T	
		A	TA	
		C	TT	

Region VIII

217	218	219	220
CCA	GAC	AAA	AAA
	T	G	G
		C	
		G	

Fig 2a

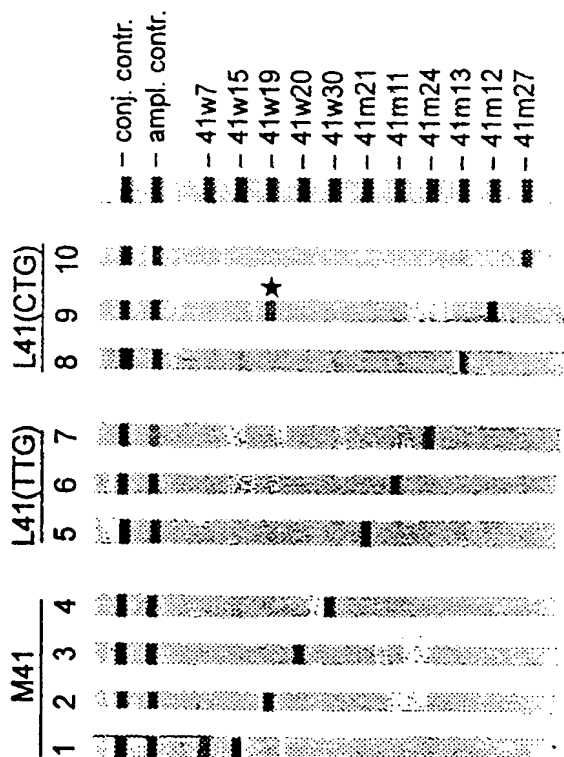


Fig 2b

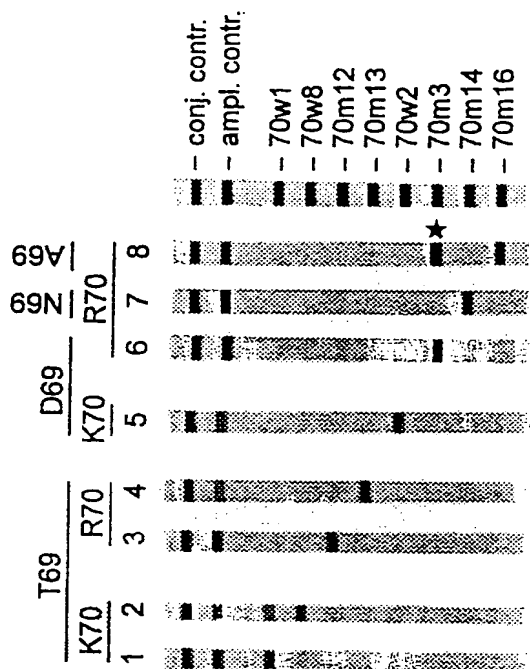


Fig 2c

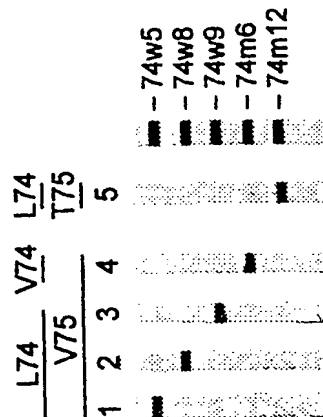


Fig 2d

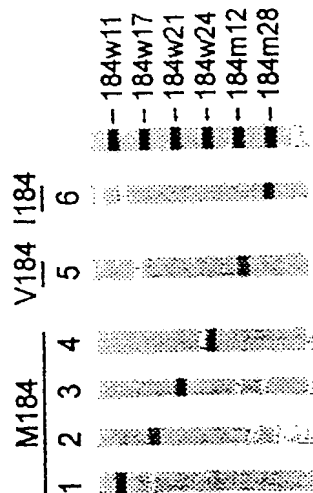


Fig 2f

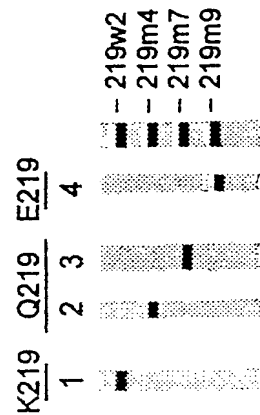


Fig 2e

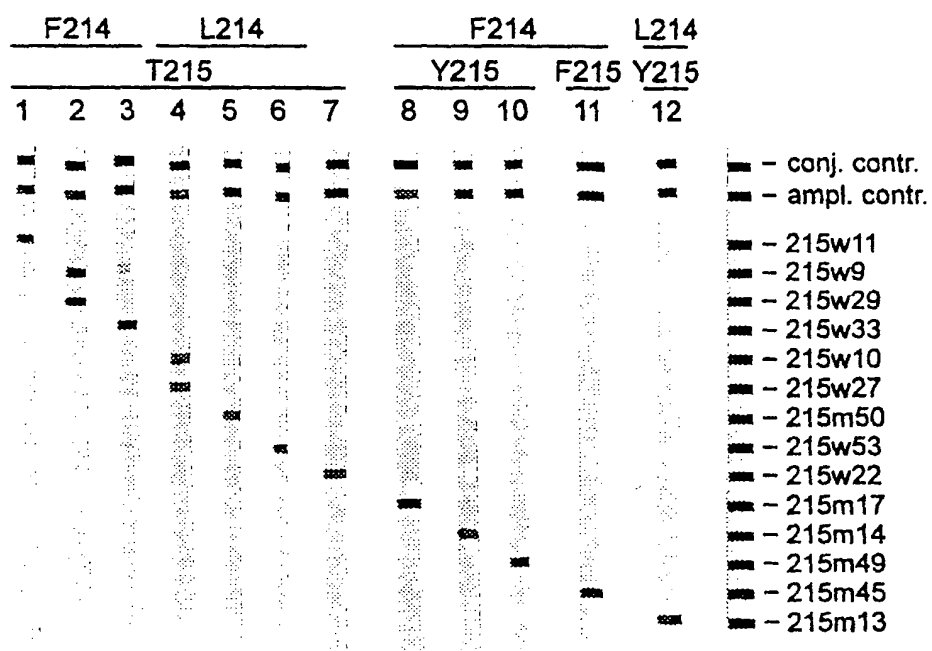
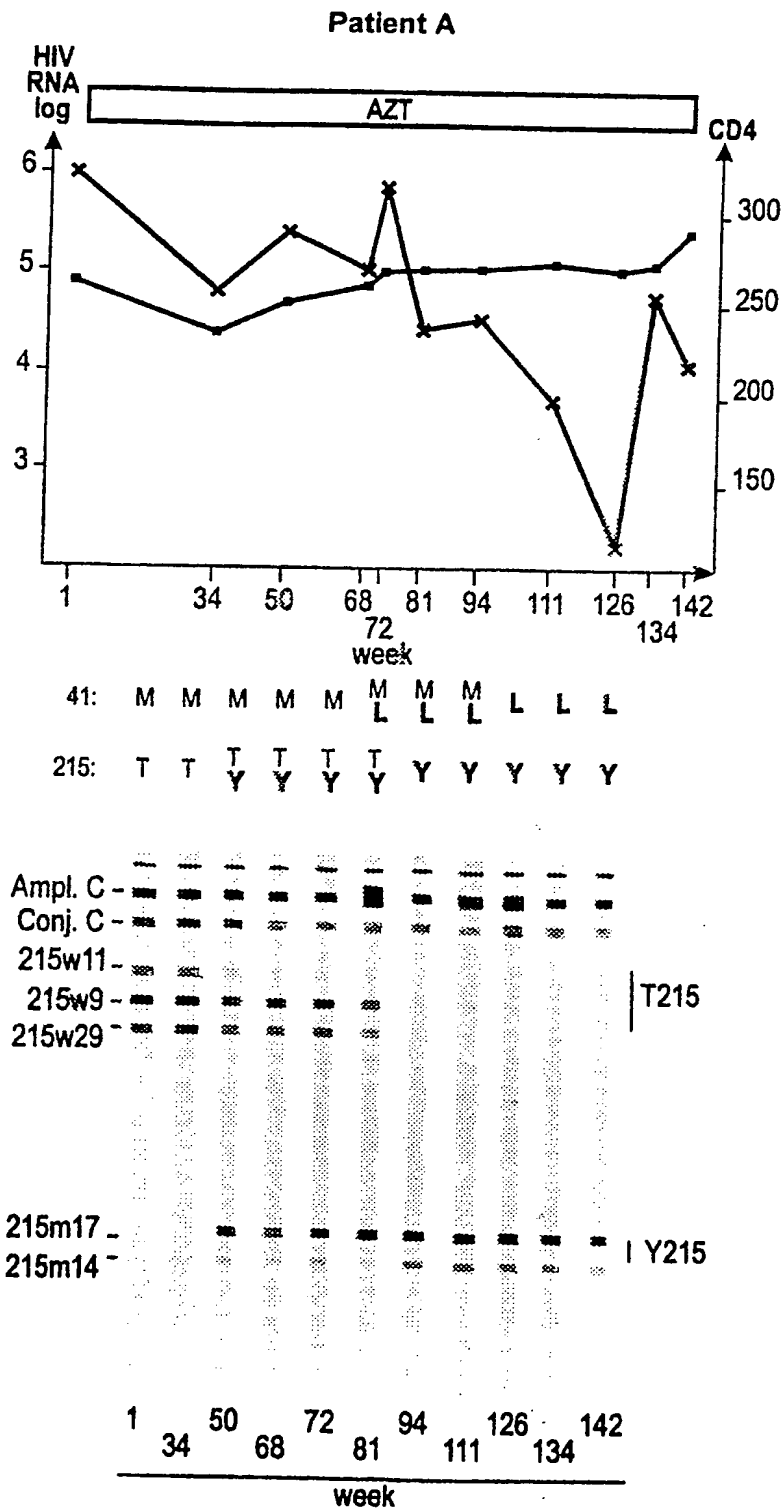
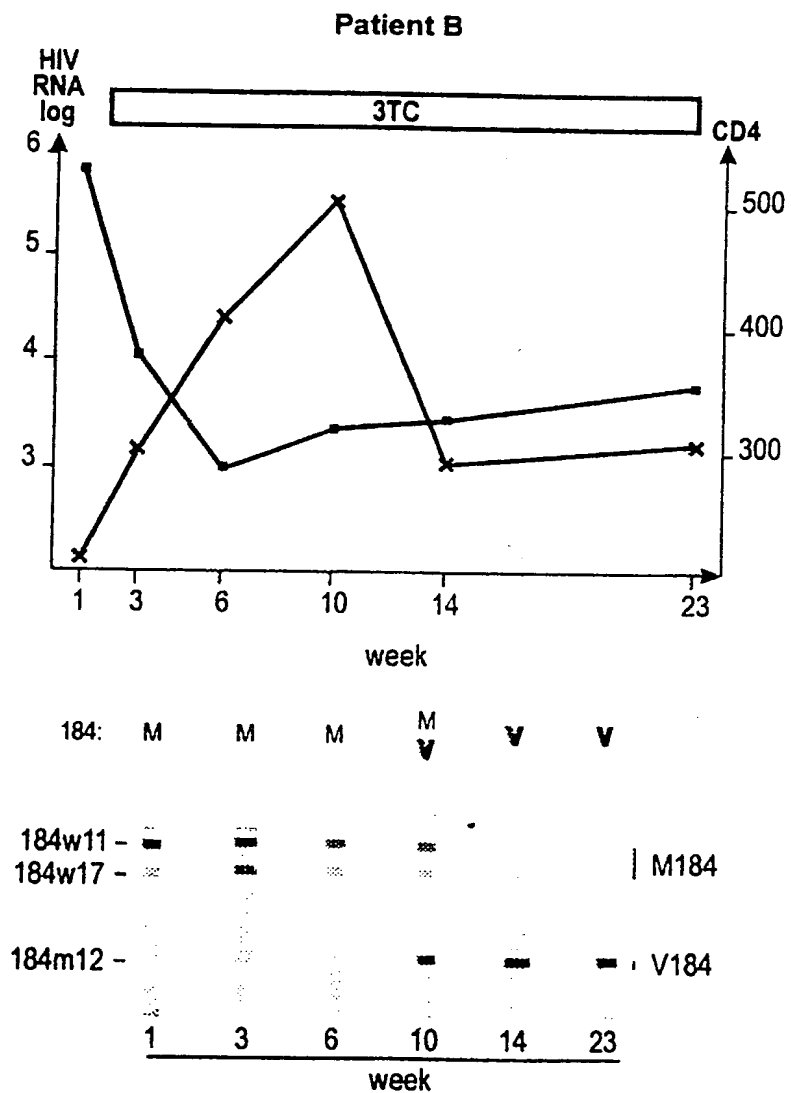


Figure 3



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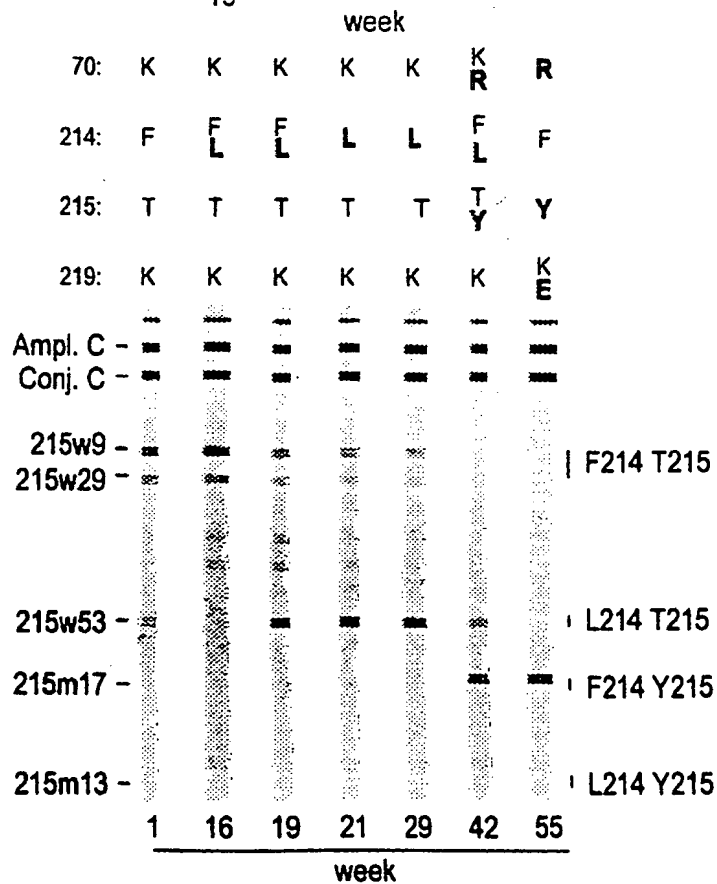
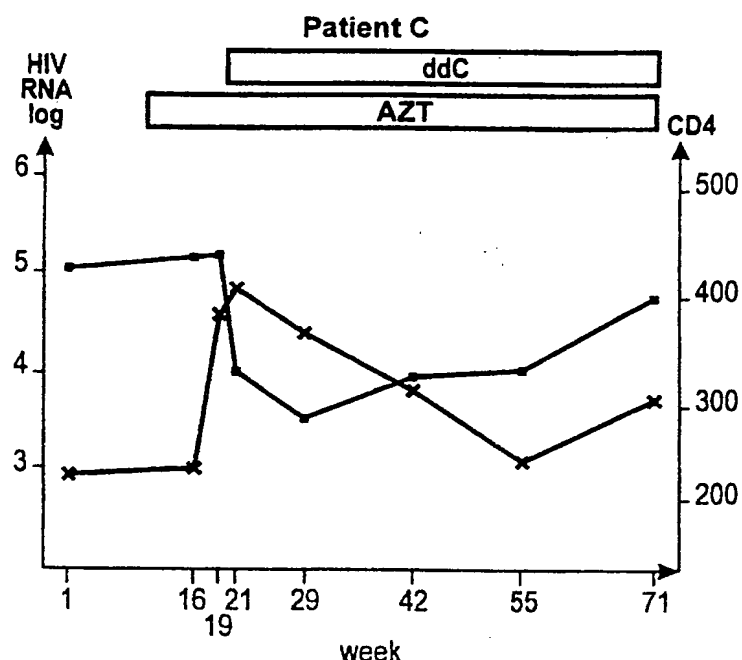




Figure 4

